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EFFECT OF CERTAIN SPECIES OF FUSARIUM ON THE COMPOSITION OF THE POTATO TUBER[1]

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INTRODUCTION

Potato tubers (*Solanum tuberosum*) are subject to attack by various parasitic fungi. Some of these organisms invade the tuber, kill the cells, break down the cell walls, and cause, directly or indirectly, a more or less complete disorganization of the host tissue. What constituents of the potato are most easily destroyed by the fungus and what compounds can not be utilized by it either in respiration or to build up its own tissue are of considerable interest in the study of the physiology of parasitism. It was to obtain information on the effect of some potato tuber rot fungi upon the tissues of the host plant that the present study was planned and carried out. In this investigation the effect of *Fusarium oxysporum* Schlecht. and *F. radicicola* Wollenw. on the sucrose, reducing-sugar, starch, pentosan, galactan, and crude-fiber content of the potato was studied. Some experiments were duplicated also with *F. coeruleum* (Lib.) Sacc.

The three species of Fusarium just mentioned are all parasites on the potato tuber. Smith and Swingle (9)[2] considered *F. oxysporum* to be the cause of a serious rot of potato tubers. Wollenweber (10) did not agree with these writers, and contended that this fungus, while the cause of a wilt disease of the potato plant, was not able to rot the tubers. This conclusion of Wollenweber's has recently been disproved by Carpenter (4), who corroborates the findings of Smith and Swingle on this point. With this species and with *F. radicicola*, the latter considered by Wollenweber and by Carpenter to be the cause of a tuber-rot of considerable importance, the writer experienced no difficulty in obtaining well-rotted tubers in two to three weeks after inoculation.

[1] The work described in this paper was carried out in cooperation with the Office of Cotton and Truck-Crop Diseases. The writer thanks Mr. C. W. Carpenter, of that office, for cultures of the fungi used.

The writer's thanks are also due Mr. A. A. Riley, of the Office of Plant Physiological and Fermentation Investigations, for assistance in the experimental part of this study.

[2] Reference is made by number to "Literature cited," p. 196.

EXPERIMENTAL METHODS

As the methods for sterilizing, sampling, and inoculating followed in this study were similar to those outlined in a study of the brownrot of the peach (7), they will be discussed here only briefly. The sterile tubers were sliced longitudinally into four parts with a flamed knife. Particular attention was given to obtaining portions of approximately the same weight and same proportionate amount of cortex and pulp. Each quarter was placed in a small wide-mouthed flask or large test tube which had been stoppered with cotton, sterilized, and weighed. The containers with the portions of potatoes were weighed again and the samples were ready for inoculation. Two of the quarters from each potato were inoculated from stock cultures of some one of the fungi used in these experiments and a small quantity of sterile water was added to each of the four containers. The four samples, two inoculated and the two corresponding control samples, were placed side by side at room temperature until the inoculated portions were well rotted. They were then prepared and analyzed. The difference between the sound and the rotted portions in the content of the compounds determined was considered to be due to the action of the fungus. All control portions infected at the time the samples were prepared for analysis and all inoculated portions infected with organisms other than those used in the inoculations were discarded.

All samples were prepared for analysis by cutting them into very thin slices with a sharp knife and washing them into the proper vessel. Precautions were observed, of course, that none of the juice or pulp should be lost. The methods of analysis for agricultural chemists[1] were usually followed in the determination of the various compounds. The sugars were extracted from the tissue with alcohol and determined as in the work on the brownrot of the peach. The method of extraction is the alcohol method of Bryan, Given, and Straughn (3), somewhat modified to suit the conditions of the experiment. The amount of cane sugar was in all cases calculated from the reducing power of the extract before and after inversion with acid.

The starch determinations in the preliminary experiments were made only by the direct acid-hydrolysis method using the finely ground potato which had been extracted with alcohol. In the work with the sound and the rotted portions of the tubers, series of analyses were also made by the diastase method with subsequent acid hydrolysis.[1] Tollen's phloroglucid method[1] was followed in all cases in the determination of the pentosans. The methyl pentosans were determined according to the method of Ellett and Tollens (6), by extracting the precipitated phloroglucid with alcohol. The galactans and the crude fiber were de-

[1] Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr., Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

termined by the usual methods[1] in dry ether-extracted samples which had been ground. For the percentage of dry matter the sliced-up samples were placed in glass-stoppered weighing bottles and covered with alcohol. The alcohol was then driven off and the samples dried to constant weight. All data were calculated to the original wet weight of the potato used. The potatoes used in the experiment were smooth white potatoes usually purchased at the local market. The cultures of fungi used in the experiments were subcultures from Carpenter's cultures of *F. oxysporum* 3395 and 3315; *F. radicicola* 3113 and 3319, and *F. coeruleum* 3501.

EXPERIMENTATION

To determine the amount of variation in content of the different compounds in the four quarters of the potato, series of preliminary analyses were carried out. In these the potato was sampled in the usual way, except that the portions were sliced immediately and prepared for analysis. The results of these analyses are shown in Tables I to VI.

TABLE I.—*Reducing sugar and sucrose content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Reducing sugar.				Sucrose.			
	Quarter A.	Quarter B.	Quarter C.	Quarter D.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
43	0.10	0.11	0.09	0.11	0.04	0.02	0.04	0.02
44	.06	.06	.07	.06	.04	.04	.03	.03
46	.17	.14	.14	.19	.02	.03	.04	.04
49	.02	.02	.03	.02	.03	.03	.03	.04
87	0	0	0	0	.07	.07	.07	.06
88	0	0	0	0	.06	.05	.06	.05
105	0	0	0	0	.05	.08	.07	.06

TABLE II.—*Starch content of quarters of sound potatoes determined by the direct acid-hydrolysis method*

[Expressed as percentage of starch, wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
71	17.88	19.52	17.07	17.26
70	16.43	15.35	15.04	16.04
47	15.04	16.04	16.00	14.50
50	18.56	17.16	16.54	17.27

TABLE III.—*Pentosan content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
19	0.51	0.43	0.46	0.48
28	.37	.35	.35	.37
47	.41	.40	.37	.40
207	.51	.48	.48	.51

[1] Wiley, H. W., ed. Op. cit.

TABLE IV.—*Galactan content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
118	0.025	0.030	0.034	0.027
135	.028	.020	.027	.029
120	.034	.024	.030	.025

TABLE V.—*Crude fiber content of quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
102	0.50	0.59	0.48	0.47
210	.47	.47	.47	.44
211	.40	.41	.39	.36

TABLE VI.—*Dry matter in the quarters of sound potatoes*

[Expressed as percentage of wet weight]

Potato No.	Quarter A.	Quarter B.	Quarter C.	Quarter D.
118	20.86	19.96	21.95	17.78
119	20.70	20.96	19.16	19.77
120	19.73	20.23	21.42	20.73
138	24.12	24.58	25.36	24.80
143	24.19	22.37	24.81	22.82

Tables I to VI show that there is considerable variation in the percentage of some of the compounds in different quarters of the same tuber, though usually the actual difference is not great. It is noticeable that two portions of the same tuber are more nearly alike in composition than samples from different potatoes. The method, therefore, which involves the comparison of the content of two quarters of the same potato is more accurate than one based on a comparison of the composition of two different potatoes. The experiments in which sound and rotted quarters were analyzed to determine the effect of the fungi upon the potato show that data from which definite conclusions may be drawn can be obtained by this method.

Inasmuch as the mycelium of the fungi was present in the rotted portions of the potatoes, it was of interest to determine what influence the compounds elaborated by these fungi would have on the apparent composition of the tuber. Quantities of mycelia of the two fungi *F. radicicola* and *F. oxysporum* were accordingly grown on potato extract. This medium was prepared by boiling sliced potatoes until they were soft, filtering the extract through cotton, and sterilizing it in suitable flasks.

The flasks of this medium were inoculated and the fungi allowed to grow for two or three weeks. The mat of mycelium was then removed, washed, dried, ground, and analyzed. The data obtained from these analyses, calculated as percentage of the dry weight, are given in Table VII.

TABLE VII.—*Amount of alcohol-insoluble substance reducing Fehling's solution when hydrolyzed with dilute hydrochloric acid, pentosans, methyl pentosans, galactans, and crude fiber in mycelium of Fusarium oxysporum and Fusarium radicicola*

[Expressed as percentage of dry weight]

Species.	Alcohol-insoluble substance reducing Fehling's solution when hydrolyzed with dilute hydrochloric acid (as dextrose).	Pentosans.	Methyl pentosans.	Galactans.	Crude fiber.
Fusarium oxysporum	34.58	2.53	0.73	0.86	21.8
	31.90	2.60	.68	.66	18.4
Fusarium radicicola	31.63	1.20	1.50	.72	20.3
	31.48	1.20	1.50	.64	17.6

It is apparent from Table VII that the fungi growing on the culture media prepared from potatoes produce pentosans, methyl pentosans, galactans, and a considerable quantity of substance which is insoluble in alcohol and reduces Fehling's solution when hydrolyzed with dilute hydrochloric acid. That this last-mentioned substance can not result from the hydrolysis of the pentosans is evident from the relatively small pentosan content of the mycelium. The amount of substance which is considered as crude fiber in the table is also quite marked. It is evident, then, that both fungi build compounds which may be expected to raise the content of pentosans, galactans, and other substances in the tissue of the potato when the fungi and host are analyzed together. It must be remembered, however, that the percentages given in Table VII are related to dry weight of washed fungus mycelium and that the content of mycelium in 25 gm. of wet weight of the potato rotted with either of these fungi would be small.

The general appearance of the rotted portion of potato was typical for tubers rotted with these fungi at laboratory temperatures (from 20° to 25° C.) in a saturated atmosphere—that is, it was a wetrot (4, p. 187). The skin apparently was uninjured and could have been removed entire in most cases. The inner portion was soft and generally disorganized. Microscopic examination showed that the cells of the interior were apparently free from each other, as if the middle lamellæ had been dissolved. The starch grains did not appear to have been eroded in the time allowed for the experiment. The method of preparing the quarters of potato for analysis has been described.

The starch and sugar determinations were usually made on the same portion by extracting the pulp with alcohol, the extract being used for

the sugar and the solid residue for the starch determinations. The effect of three species of Fusarium, *F. oxysporum*, *F. radicicola*, and *F. coeruleum*, on the starch and sugar content of sound and rotted quarters of the same tubers was studied. The data obtained from the determination of the sugars are shown in Table VIII.

TABLE VIII.—*Reducing sugar and sucrose content of the sound and rotted quarters of potatoes*

[Expressed as percentage of the original wet weight]

Species of Fusarium and potato No.	Reducing sugar.		Sucrose.	
	Rotted quarter.	Sound quarter.	Rotted quarter.	Sound quarter.
Infected with *Fusarium oxysporum:*				
160	0.04	0.31	0.10	0.66
159	.04	.28	0	.67
158	0	.44	0	1.03
Infected with *Fusarium coeruleum:*				
149	.13	.40	.12	.39
150	.04	.47	.24	.59
151	.17	.37	0	.66
Infected with *Fusarium radicicola:*				
32	0	.03	.04	.24
26	0	.02	.04	.19
34	0	.03	.02	.09
41	0	.02	0	.42

In Table VIII it may be seen that all three species of Fusarium used the sugars. In most cases practically all the sugar had disappeared from the rotted portion, the cane sugar being utilized almost if not quite as completely as the reducing sugars. That the fungi could use disaccharids directly—that is, without breaking them down to their constituent monosaccahrids—seemed unlikely. It was therefore probable that the fungi secreted enzyms which were capable of hydrolyzing cane sugar, and possibly maltose also. To determine this point, tests were made for sucrase and maltase in extracts of the mycelium of *F. oxysporum* and *F. radicicola*. The fungi were grown for about three weeks or until a thick mat of mycelium was formed on potato extract. The felt was then separated from the liquid, ground up in a mortar and digested for 48 hours under toluol. The extract was filtered off and portions of it added to solutions of the sugars of known concentration. Controls of the boiled extract were also prepared. After the preparations had been allowed to stand overnight at laboratory temperature the amount of reducing sugar was determined. It was found that in the preparations of unboiled extract the sugars, both sucrose and maltose, were inverted almost quantitatively. The boiled extracts were practically without effect. It is evident then that the two fungi secrete both sucrase and maltase.

The starch determinations in the sound and rotted portions of the same tuber were made by two methods, as has been said. The data obtained by the direct acid hydrolysis method are given in Table IX, while the results of the determinations by the diastase method with subsequent acid hydrolysis are shown in Table X.

TABLE IX.—*Starch content of sound and rotted quarters of potatoes infected with different species of Fusarium, as found by direct acid hydrolysis*

[Expressed as percentage of starch of the original wet weight]

Potato No.	*Fusarium oxysporum.*		Potato No.	*Fusarium coeruleum.*		Potato No.	*Fusarium radicicola.*	
	Rotted quarter.	Sound quarter.		Rotted quarter.	Sound quarter.		Rotted quarter.	Sound quarter.
165.....	14. 40	13. 53	149.....	19. 19	18. 69	34.....	16. 18	15. 79
168.....	14. 08	14. 67	150.....	18. 72	17. 48	26.....	15. 24	16. 60
180.....	16. 16	14. 62	151.....	22. 06	22. 22	32.....	15. 15	16. 01
						41.....	16. 83	16. 85

TABLE X.—*Starch content of sound and rotted quarters of potatoes infected with different species of Fusarium as determined by the diastase method with subsequent acid hydrolysis*

[Expressed as percentage of starch, wet weight]

Potato No.	*Fusarium oxysporum.*		Potato No.	*Fusarium radicicola.*	
	Rotted quarter.	Sound quarter.		Rotted quarter.	Sound quarter.
25.....	17. 77	16. 85	47.....	17. 50	16. 66
33.....	12. 50	11. 32	48.....	16. 66	15. 16
27.....	15. 32	14. 05			

The effect of the fungi upon the starch in the potatoes is in marked contrast to their action on the sugars. In Table IX, which gives the results of starch determinations by the direct acid-hydrolysis method, it may be seen that the starch content of the rotted portion appears to be higher in many cases than that of the corresponding sound quarter. In the determinations by the diastase method followed by acid hydrolysis the apparent starch content of the rotted portion is always higher, as shown in Table X. The fact that the fungi build up substances which are insoluble in alcohol and reduce Fehling's solution when hydrolyzed with dilute hydrochloric acid, as shown in Table VIII, would account for any apparent increase in starch content in the rotted portion when the starch is determined by the direct acid-hydrolysis method. If the substances are also either soluble in hot water originally or made so by the diastase treatment, the apparent increase in starch content when the starch is determined by this method would be explained. In the diastase method the starch paste is liquefied by the action of the diastase,

then filtered, and the filtrate hydrolyzed with dilute hydrochloric acid. Some of the mycelium of these fungi was extracted with alcohol, and then dried and extracted with hot water. The extract was then filtered off, treated with hydrochloric acid exactly as in the acid hydrolysis of starch, neutralized and tested for reducing substances. A considerable quantity was found. The filtrate did not give a qualitative test for pentosans. The apparent increase in starch content in the rotted portions of the potatoes, then, is due to compounds laid down by the fungi. From the fact that only a small amount of mycelium of these fungi could be present in the rotted potato it would seem probable that if the starch were attacked to any extent the apparent starch content as obtained by acid hydrolysis would be lowered in all cases. To obtain further information on this point experiments were carried out to ascertain whether these fungi secreted diastase and if so, whether this enzym could break down the starch grains of the potato.

Extracts of the undried, ground mycelium of the two fungi, *F. oxysporum* and *F. radicicola*, were made with 50 per cent glycerin. These extracts were filtered after 24 hours through absorbent cotton and portions added to a 2 per cent solution of "soluble starch." Suitable controls were prepared and all preparations allowed to stand in an incubator under toluol at 30° C. for 48 hours. At the end of this time the starch was practically all broken down by the extracts of both fungi. Similar experiments were carried out with starch paste made from potato starch with positive results. The fungi then secrete diastatic enzyms. The experiments, however, did not prove that the diastases were able to attack the starch grains before they were broken down. Brown and Morris (2) have shown that malt diastase can not act on ungelatinized potato starch, though the starch grains of barley are readily eroded by it. Whether the enzyms in the extracts of the mycelium could erode the starch grains of the potato at room temperature was determined by placing some well-washed potato starch in extracts and allowing the preparations to stand under toluol. They were shaken up and examined from time to time, but no sign of erosion of the starch grains was evident even at the end of a week. The extracts used were tested on starch paste or "soluble starch" with positive results. Smith and Swingle (9) mention that the starch in the potatoes rotted with *F. oxysporum* was apparently not eroded. It is, of course, possible that the potato starch grains are very slowly attacked by the diastases of these fungi or that some inhibitor is present which prevents the action of the enzym on the starch in this condition at the temperature at which these studies were made. These points should be investigated. At present, however, the conclusion seems warranted in view of the evidence that the starch of the potato is not appreciably affected by the fungi.

From the fact that these fungi penetrate the cell walls or parts of the cell walls of the potato in living parasitically upon their host, their effect

on the constituents of the cell wall was considered of especial interest. The substances studied in this investigation which may be considered to be, in part at least, components of the cell walls are the pentosans, crude fiber, and galactans (5). Inasmuch as the fungi apparently do not affect the skin in rotting the potato, it was considered of interest to find out the relative distribution of the pentosans and crude fiber in the skin and inner portion of the potato. For these analyses the potatoes were peeled as thinly as convenient and determinations made on the weighed peeling and inner portion separately. The results of the pentosan determinations are given in Table XI.

TABLE XI.—*Pentosan content of the peeling and inner portion of potatoes*

[Expressed as percentage of pentosans, wet weight]

Potato No.	Skin.	Inner portion.	Potato No.	Skin.	Inner portion.
116	0.62	0.28	140	1.02	0.59
133	.88	.39	163	.72	.36
134	.80	.47	164	1.07	.50

When the pentosan content is calculated as wet weight, it is about half as great in the inner portion of the tuber as in the skin. There is, nevertheless, a considerable amount of the furfurol-yielding compounds in the fleshy part of the potato. Inasmuch as the fungus has practically no effect on the skin, it is to be considered that practically all changes in the pentosan content that take place during rotting are in the inner portion of the tuber. The results obtained from the pentosan determinations on the sound and the rotted portions of the potato tubers are shown in Table XII.

TABLE XII.—*Pentosan and methyl-pentosan content of sound and rotted quarters of potatoes*

[Expressed as percentage of pentosans, wet weight]

Potato No.	Sound quarter.			Rotted quarter.		
	Total pentosans.	Pentosans.	Methyl pentosans.	Total pentosans.	Pentosans.	Methyl pentosans.
Infected with *F. oxysporum:*						
29	0.53	0.47	0.06	0.50	0.35	0.15
30	.53	.41	.12	.46	.35	.11
35	.45	.36	.09	.44	.35	.09
40	.52	.42	.10	.37	.26	.11
Infected with *F. radicicola:*						
171	.28	.23	.05	.25	.20	.05
174	.37	.32	.05	.29	.24	.05
176	.25	.19	.06	.26	.21	.05

Table XII shows that the total pentosan content, which includes all furfurol-yielding matter, and the pentosan content, which is the total pentosan content after the methyl pentosans have been extracted, are higher in all but one instance in the sound portions of the tuber. There is slightly more variation in methyl pentosan content; it is the same or greater in the rotted as in the sound portion in all but two cases. The fungi evidently use the pentosans, but do not affect the methyl pentosans to any extent. It is to be remembered that these fungi build up both pentosans and methyl pentosans when growing on potato extract. The content of these substances, then, in the rotted portions given in Table XII is undoubtedly the difference between the amount of pentosans broken down by the fungi in the interior of the potato and the amount built up by the fungi. The destructive processes evidently proceed more rapidly than the constructive, and some of the pentosans of the potato are used either in respiration or in the building up of other compounds.

From the effect of the fungi on pentosans it was considered probable that enzyms which could hydrolyze these compounds were present in the mycelium. Experiments were undertaken to determine this point.

The experiments were carried out as described in a previous paper (8), except that the fungi were grown on potato extract instead of a synthetic medium with gum arabic as a source of carbon. Xylan from rye straw was used as a substrate. The results of these experiments are given in Table XIII.

TABLE XIII.—*Effect of boiled and unboiled extract of mycelium upon xylan from rye straw, as shown by alcohol-soluble furfurol-yielding material and substance reducing Fehling's solution. (0.2 gm. of xylan in each preparation was maintained at 30° C. for one week.)*

Species of Fusarium.	Quantity of cuprous oxid derived from material reducing Fehling's solution.		Quantity of alcohol-soluble furfurol-yielding substances as pentosans.	
	Unboiled.	Boiled.	Unboiled.	Boiled.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
Fusarium radicicola	45.2	15.4	13.1	5.7
	44.8	14.8	13.1	5.7
Fusarium oxysporum	14.1	6.5	18.6	6.8
	16.4	6.5	14.6	6.8

It is evident from Table XIII that the extracts of the fungi are able to break down xylan prepared from rye straw to an alcohol-soluble compound which reduces Fehling's solution and which forms furfurol when boiled with hydrochloric acid. The fungi then secrete an enzym or enzyms which can break down xylan probably to xylose.

The crude fiber of the potato is undoubtedly a mixture of compounds, among which are some of the cell wall constituents, including whatever

cellulose may be present. The distribution of the crude fiber throughout the tuber is not as uniform as that of the pentosans, as is shown by a comparison of Tables XI and XIV.

TABLE XIV.—*Crude fiber content of the skin and inner part of the potato tuber*

[Expressed as percentage on both a wet weight and dry weight basis]

Potato No.	Percentage of crude fiber, wet weight.		Percentage of crude fiber, dry weight.	
	Skin.	Inner portion.	Skin.	Inner portion.
210	1.54	0.25	11.11	1.16
211	1.33	.25	6.61	1.06
212	1.20	.36	7.89	1.82

From Table XIV it may be seen that the crude-fiber content of the peeling is 3½ to 6 times greater than that of the inner portion calculated on a wet weight basis and from 4 to 10 times greater on the basis of dry weight. The inner portion of the potato contains usually a lower percentage of crude fiber than of pentosans.

The determinations of crude fiber on the sound and rotted portions of the potato tubers are given in Table XV.

TABLE XV.—*Crude-fiber content in sound and rotted quarters of potatoes*

[Expressed as percentage of wet weight]

Rotted with *Fusarium radicicola.*			Rotted with *Fusarium oxysporum.*		
Potato No.	Rotted quarter.	Sound quarter.	Potato No.	Rotted quarter.	Sound quarter.
37	0.56	0.54	177	0.71	0.58
39	.57	.56	178	.73	.62
115	.40	.37	179	.69	.62

The crude-fiber content is always higher in the rotted quarter of the tuber than in the corresponding sound portion, though the difference is not great. As has been mentioned earlier in this paper, the fungus builds up a considerable quantity of substance which is not dissolved in either the acid or alkali used in the crude-fiber determination; to this is due the rise in the crude-fiber content of the potato during rotting. It is possible, of course, that the fungi may break down the crude fiber of the host plant and build up some similar substance with greater rapidity. From the evidence brought out in these experiments, then, it is impossible to draw definite conclusions.

The substances in the potato which give mucic acid when boiled with proper concentration of nitric acid are considered in this study as galactans. They are present in small quantities in the potato, and the com-

bination in which they occur in the tuber was not investigated. Galactose might occur in combination with raffinose, in a glucoside or combined in the cell walls. It probably occurs in plants most commonly in the last-mentioned combination. The effect of the fungi upon the galactan content of the potato is shown in Table XVI.

TABLE XVI.—*Galactan content of sound and rotted quarters of potatoes*

[Expressed as percentage of wet weight]

Rotted with *Fusarium radicicola*.			Rotted with *Fusarium oxysporum*.		
Potato No.	Rotted quarter.	Sound quarter.	Potato No.	Rotted quarter.	Sound quarter.
27	0.039	0.062	166	0.069	0.071
31	.033	.060	167	.068	.076
42	.029	.030	172	.081	.083

It is evident from the table that the fungi lower the galactan content of the potato. The fungi produce galactans when growing upon potato extract and the data in Table XVI show that the breaking down process proceeded faster than the building up.

The amount of dry matter of the sound and rotted quarters determined as mentioned earlier in this paper is shown in Table XVII.

TABLE XVII.—*Amount of dry matter in sound and rotted quarters of potatoes*

[Expressed as percentage of wet weight]

Rotted with *Fusarium radicicola*.			Rotted with *Fusarium oxysporum*.		
Potato No.	Rotted quarter.	Sound quarter.	Potato No.	Rotted quarter.	Sound quarter.
27	20.83	21.19	166	17.73	18.91
31	19.88	22.59	167	18.93	20.45
42	20.98	22.13	172	18.17	19.36

As was to be expected, the rotting of the potato by the fungi lowered the percentage of dry weight as calculated to the original weight of the portion of the potato used in the experiment. This is probably due to an increased respiration—that is, a respiration of the quarter of the potato plus the respiration of the fungus which in a given time is greater than a portion of the same potato alone.

DISCUSSION

From the foregoing pages it is evident that the tuber-rot fungi used in this study considerably alter the composition of the potato. That they should be able to utilize the sugars of the potato was to be expected. Most fungi use glucose readily as a source of carbon. Behrens (1) has shown that *Sclerotinia fructigenia* lowers the sugar content of apples in

rotting them. The brownrot fungus of peaches reduces the sugar content of that fruit. The presence of the enzyms sucrase and maltase in fungi has frequently been recorded.

The starch content of the potato makes up the greater part of its dry weight and may be regarded as stored food material. That the fungi which so efficiently utilize the monosaccharids and disaccharids of the potato tuber are unable, apparently, to affect this polysaccahrid is of considerable interest. The fungi grow for the most part in the cell walls and thus are not closely in contact with the starch grains. This might retard the action because of the low rate of diffusion of the diastase but could hardly inhibit it entirely. The fact that the diastases of these fungi had no apparent effect on unbroken starch grains *in vitro* during the time of the experiment, while potato starch when gelatinized was readily hydrolyzed by these enzyms, indicates that the rate of action under what are usually favorable conditions for such reaction is to say the least very low. The experiments seem to show that enzymic studies are of doubtful value in determining the effect of the parasite on the host plant unless corroborated in a study of the physiological relations existing between the two organisms. The effect of the fungi on the pentosan and galactan content of the potato shows that they can break down at least some of the constituents of the cell wall. Now, when a parasitic fungus such as those used in this study enters a cell of its host plant, it must either force its way in mechanically by exerting sufficient pressure to rupture the cell wall or a portion of the cell wall must be dissolved. Likewise, in growing between the cells of the host plant where no appreciable intercellular spaces exist, the cells must be forced apart mechanically or some parts of the cell walls dissolved. It is evident from their effect on the pentosans that these fungi are able to dissolve at least some portions of the cell wall. That they secrete enzyms which can hydrolyze xylan is more evidence on this point. The crude-fiber content of the potato was increased in rotting owing to the formation in the fungi of some substances which were not broken down by the acid or alkali treatment in the crude-fiber determinations. Therefore it was impossible to obtain evidence as to the effect of the fungi upon the crude fiber. As shown in the tables the crude-fiber content of the inner portion of the potato is not high. It is noticeable that throughout this study the different species of Fusarium had practically the same effect on the potato.

CONCLUSION

In conclusion, it has been shown in this study that the fungi in the potato reduced the content of sugar, both sucrose and reducing sugar, pentosans, galactans, and dry matter. The starch and methyl pentosans are apparently not affected appreciably, and the crude-fiber content was not reduced. It was shown that these two species of fungi secrete sucrase, maltase, xylanase, and diastase; the last-mentioned enzym is apparently incapable of acting on the ungelatinized potato starch.

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HYPERASPIS BINOTATA, A PREDATORY ENEMY OF THE TERRAPIN SCALE

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INTRODUCTION

One of the most effective enemies of lecanium scales is the coccinellid beetle *Hyperaspis binotata* Say. Its economic importance was impressed on the writer during the seasons of 1912 and 1913, when he was studying the life history and control of the terrapin scale (*Eulecanium nigrofasciatum* Pergande). Throughout the spring and early summer the larvæ, conspicuous by their flocculent covering, could be found in large numbers feeding upon the immature scales and overturning the adult scales. The adult beetles do not feed upon the mature scales, but they destroy the young and also attack aphides, or plant lice, and other soft-bodied insects. In view of the economic importance of this beetle a study of its life history was undertaken at the suggestion of Dr. A. L. Quaintance, in charge of Deciduous Fruit Insect Investigations, Bureau of Entomology. The work was begun in the summer of 1912 and completed in 1913.

HISTORICAL SUMMARY

Very little has been written about *Hyperaspis binotata*. Say (1, p. 303),[1] in 1826, described the male under the present name, and the female as *Coccinella normata*. G. R. Crotch (2, p. 380) considered the form with the subapical red spot as a variety of *H. signata* Olivier, and gave as synonyms *H. binotata* Say, *H. normata* Say, and *H. leucopsis* Melsheimer.

T. L. Casey (3, p. 124), in 1899, considered *H. binotata* Say as a distinct species and gave the following synonymy: *H. signata* Le Conte, *H. normata* Say, *H. affinis* Randall, and *H. leucopsis* Melsheimer.

J. G. Sanders (4, p. 3), in 1905, mentions *H. binotata* as a valuable predatory enemy of *Pulvinaria* spp. J. B. Smith (5, p. 606; 6, p. 570), in the same year, reported the same species as reducing an infestation of *Pulvinaria* spp. at Montclair, N. J., from 500 to 1,000 scales to a leaf to about one dozen scales to a leaf.

S. A. Forbes (7), in his annual report for 1908, mentions the species as one of the principal enemies of *Pulvinaria* spp. in Illinois. In 1910, W. S. Blatchley (8, p. 523), gives a key to the species of Hyperaspis found in Indiana and remarks that *H. binotata* Say is "a variety of *H. signata* Oliv., having the subapical spot lacking, color and structure otherwise exactly as in that species." W. E. Britton (9, 8), in 1914, treats this species,

[1] Reference is made by number to "Literature cited," p. 203.

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mentioning it as a great destroyer of the cottony maple scale (*Pulvinaria vitis* Linnaeus) and stating that it feeds upon both the woolly maple-leaf scale (*Phenacoccus acericola* King) and the tulip scale (*Eulecanium tulipiferæ* Cook).

These references bring the history of the species down to the date of the present paper, which deals with the life history and habits of the species when feeding upon the terrapin scale.

DISTRIBUTION

H. binotata occurs in most of the territory east of the Mississippi River and extends west of this river in some States to the semi-arid region. It is most abundant in the Atlantic States from Connecticut to Maryland, but is common from New Jersey to Illinois. All localities known to the writer are indicated upon the map (fig. 1).

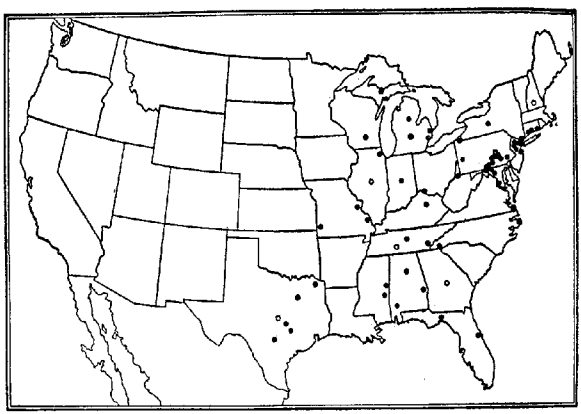

FIG. 1.—Map showing the distribution in the United States of *Hyperaspis binotata*: ●=definite record; ○=doubtful record.

HOSTS

H. binotata feeds upon honeydew, aphides, aphis eggs, and mealy bugs and other soft-bodied scales. The larvæ, so far as observed, feed upon scale larvæ and young scales. They seem to have preyed originally upon species of Pulvinaria, to the egg masses of which the larvæ have a superficial resemblance. The species thrives upon the terrapin scale and seems to be rather more abundant where it preys exclusively upon this scale.

DESCRIPTION OF LIFE STAGES

IMAGINAL STAGE

The adult (Pl. XXIV, fig. 1, 2) is a small hemispherical beetle which passes the winter in rubbish or under bark. It was described by Say (1) in 1826 from the male as follows:

"Black, lateral margin of the thorax and head yellow; each elytron with a rufous spot; body rounded-oval, convex, punctured, black, polished; head pale yellow, labrum and transverse line on the vertex piceous; thorax with a yellow margin extending for a short distance on the anterior margin; anterior margin with an obsolete yellowish line interrupted in the middle; elytron each with a rufous, orbicular, central spot."

EGG STAGE

The egg (Pl. XXIV, fig. 3), which was first obtained by the writer in 1913, is oblong-elliptical and somewhat depressed; 10 specimens measured from 0.6 to 0.775 mm. in length (average, 0.704 mm.) and from 0.218 to 0.4 mm. in width (average 0.312 mm.). In color it is light salmon, changing ultimately to ash-gray; the shell is membranous, becoming indented with age. Hatching takes place through a longitudinal slit on the upper surface.

LARVAL STAGE[1]

The first instar has characteristic markings, and represents a rather primitive type of coccinellid larva. The other instars are similar to the first, but they are covered by a white fleece of wax filaments which masks their characters.

FIRST INSTAR (Pl. XXIV, fig. 4).—Length 1.22 mm. (1.125 to 1.275 mm.), width 0.478 mm. (0.450 to 0.575 mm.); body grayish white, semiopaque, cylindrical, and tapering caudad. Head black, with a white trident spot over the epicranial and frontal sutures; three pairs of ocelli present; length 0.125 mm., width 0.225 mm. Thorax sparsely pilose, the segments each with a pair of black dots; prothorax with two black clouded areas surrounding, but mainly cephalad of the dots. Abdominal segments each with a row of eight hairs and a pair of long lateral setæ; ninth segment black above; tenth segment, the so-called anal lobe, retractile.

SECOND INSTAR (Pl. XXIV, fig. 3, *a*).—Length 2.5 mm. (1.3 to 2.75 mm.), width 1.08 mm.; body yellowish white, pubescent and covered with a white fleece. Head black with the trident spot mildly obscured; length 0.175 mm., width 0.325 mm. Thorax white, immaculate; legs gray, marked with black. Abdomen devoid of conspicuous lateral setæ.

THIRD INSTAR.—Length 2 to 3.38 mm., mostly 2.5 mm.; width 0.9 to 1.75 mm., mostly 1.125 mm. Head black, pigmentation on the posterior part of labium confluent; length 0.275 to 0.3 mm., width 0.45 to 0.5 mm., mostly 0.475 mm. Abdomen with eight pairs of conspicuous blood pores. Otherwise as in the second instar.

FOURTH INSTAR (Pl. XXV, fig. 1, 2).—Length 2.5 to 6.25 mm., mostly 5.5 mm.; width 1.125 to 2.5 mm., mostly 2.25 mm. Body subglobose, yellowish gray. Head glabrous, white, flecked with black, pigmentation on the posterior part of labium not confluent on the median line; length 0.3 to 0.375 mm., mostly 0.35 mm.; width 0.575 to 0.65 mm., mostly 0.6 mm. Otherwise as in the third instar.

PUPAL STAGE

Pupa (Pl. XXV, fig. 3, 4) inclosed within the larval skin; length 2.03 to 4.19 mm., mostly 3.9 mm.; width 1.77 to 1.86 mm.; color uniform chestnut-brown; ovate, with a depressed segmented area on the dorsum; dorsal surface hispid; ventral surface mildly pilose.

[1] A detailed morphological study of this larva by Dr. Adam Böving is in course of preparation.

HABITS AND SEASONAL HISTORY

THE BEETLES

The beetles emerge from hibernation at Mont Alto, Pa., about the middle of April and commence mating about the 20th of that month. When the species is feeding upon the terrapin scale, the beetles hibernate for the most part at the bases of scale-infested peach (*Amygdalus persica*) trees. After emerging from hibernation they soon depart in search of food and do not return to the peach until the adult scale, which the beetle is unable to destroy, begins to deposit honeydew—about the middle of May. For the rest of the season the species remains upon the peach, feeding upon the scale and its honeydew. The overwintering beetles are nearly all dead by the middle of July, while the new brood of beetles escapes from pupæ for the most part during the first half of that month.

There is some indication of a second brood, but there is not enough evidence at hand to establish it.

THE EGGS

A very typical group of four eggs just as they were deposited is shown in Plate XXIV, figure 3. It will be noticed that the eggs are not clustered, but are placed more or less at random in the irregularities of the bark adjacent to the host. The terrapin scale upon which the species was feeding is found only upon young wood, the growth rings of which supply a convenient shelter for the eggs of the beetle. It is not unusual, however, to find eggs in crevices at the base of fruit spurs or even upon smooth bark. It is worthy of note in this connection that the eggs are not placed under the scales. It was found that the membranous shell became dry and shriveled in from three to six days, and that the egg changed to an ash-gray near the end of the incubation period.

The first eggs of the season were laid upon the twigs of scale-infested peach trees at Mont Alto, Pa., on May 3, 1913, but were immediately consumed by the beetles, as were all later eggs, until the food supply became abundant. It was not until May 26 that eggs were permitted to hatch. Oviposition reached its maximum about June 5, and continued in a small way until September 1. Owing to the tendency of the beetles to devour their eggs, it was not possible to determine definitely the beginning of oviposition or the total number of eggs; 36 was the largest number obtained from a single female, but there were indications that several times that number had been deposited. Incubation lasts from six to eight days; the average for 18 eggs deposited between June 27 and 30, 1913, was seven days.

THE LARVÆ

The larvæ at the time they escape from the egg have the pigment lacking from the head, legs, and ninth abdominal segment. They begin searching at once for the terrapin scales; and when one is found, a larva enters the brood chamber through the anal cleft, where it remains during the first and second instars. The first noticeable appearance of the coccinellid larvæ in the orchard, which occurs about June 18, coincides with the beginning of reproduction of the terrapin scale. Once within the brood chamber of a scale the coccinellid larva (Pl. XXIV, fig. 4) preys upon the new-born young of that particular scale until the end of the second instar, by which time the rapidly growing coccinellid displaces the scale.

The second molt is made in the open, mostly at the base of a fruit spur. In the third and fourth instars many mature scales are destroyed, being displaced (Pl. XXIV, fig. 5) by the coccinellid larvæ as these thrust their heads into the brood chambers to secure the young scales. When all the old scales have been destroyed, the ladybird larvæ, which now have a superficial resemblance to mealy bugs, migrate to the leaves and there continue to feed upon such of the scale larvæ as were able to reach the leaves. It is estimated that a single coccinellid larva will destroy 90 mature scales and 3,000 larvæ.

The length of the larval instars, together with the number of specimens used in their determination, is shown in Table I.

TABLE I.—*Length of the larval instars of Hyperaspis binotata*

Instar.	Number of specimens.	Length of instar.		
		Average.	Minimum.	Maximum.
		Days.	*Days.*	*Days.*
First	17	2.98	2	4
Second	11	2.18	1	3
Third	7	2.71	2	4
Fourth	5	12.00	12	12

The dates at which the respective instars occur in the field are given in Table II. The first and second dates show the time of greatest abundance; the first and last dates show the total time of occurrence for each instar.

TABLE II.—*Sequence of the seasonal appearance of the larval instars of Hyperaspis binotata in the field*

Instar.	Date present in field.
First	June 17 to 20 to Sept. 15.
Second	June 20 to 22 to Sept. 20.
Third	June 22 to 25 to Sept. 25.
Fourth	June 25 to July 7 to Sept. 30.

The author has depended upon head measurements in distinguishing the instars; a key for this purpose (Table III) has proved satisfactory. As will be seen from the table, it is only necessary to consider the width of the head.

TABLE III.—*Key for determining the larval instars of Hyperaspis binotata according to width of head*

Instar.	Width of head.
	Mm.
First	0.225
Second	.325
Third	.475
Fourth	.600

THE PUPA

The pupal period lasts for from 10 to 13 days, averaging 12 days. Pupæ appear in the field early in July and are most abundant from the 7th to the 20th of the month. They are found, surrounded by the last larval skin, attached to leaves or concealed in clusters under bark. An occasional one may be found as late as October.

NATURAL ENEMIES

There seem to be very few enemies of this ladybird. No parasites were obtained, and no birds were observed to feed upon it. Aphis lions were found preying upon the eggs, and a common plant bug, *Brochymena* sp., was taken upon two occasions with this coccinellid impaled upon its beak.

SUMMARY

Hyperaspis binotata Say is found in the eastern United States and westward to the semiarid region. It feeds upon aphides and soft-bodied scales and is very effective in controlling the cottony maple scale and the terrapin scale. The eggs are salmon-colored and are deposited singly on twigs adjacent to the hosts. The life cycle requires 39 days and is as follows: Incubation, 7 days; first instar, 3 days; second instar, 2 days; third instar, 3 days; fourth instar, 12 days; pupa, 12 days.

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PLATE XXIV

Hyperaspis binotata:

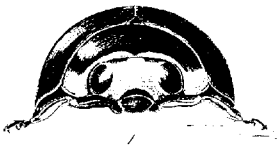
Fig. 1.—Male, showing the characteristic markings. Much enlarged.

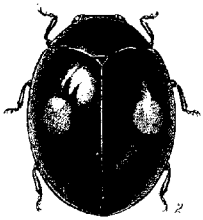
Fig. 2.—Female, showing the dorsal view. Much enlarged.

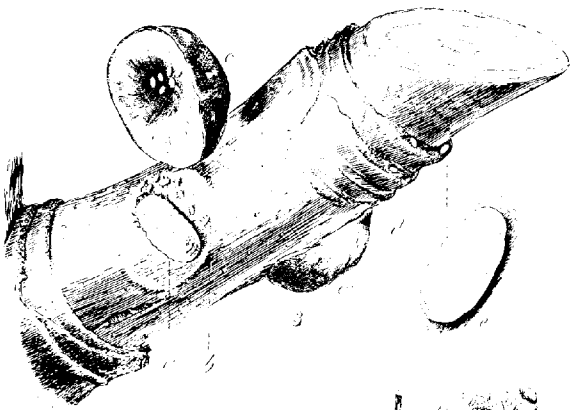
Fig. 3.—Eggs and a second-instar larva. *a*, Second-instar larva as disclosed by displacing the host; *b*, larvæ of the terrapin scale, *Eulecanium nigrofasciatum*; *c*, a displaced scale; *d*, eggs "in situ"; *e*, egg somewhat enlarged.

Fig. 4.—First-instar larva.

Fig. 5.—Method of attacking the mature scales during the third and fourth instars.

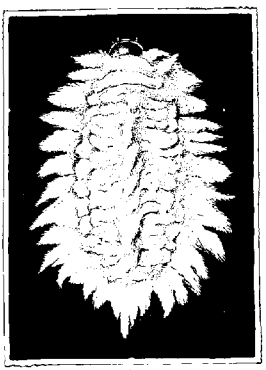






Hyperaspis binotata

PLATE XXV



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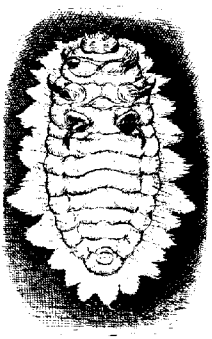

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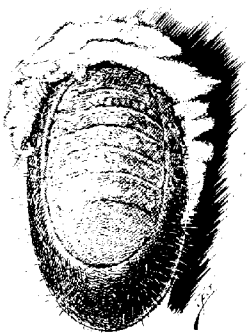

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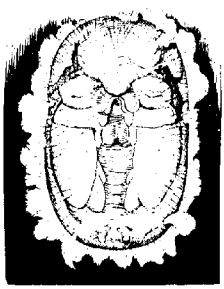

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PLATE XXV

Hyperaspis binotata:

Fig. 1.—Mature larva as it appears when attacking the leaf-attached larvæ of the terrapin scale, *Eulecanium nigrofasciatum*.

Fig. 2.—Ventral view of mature larva.

Fig. 3.—Dorsal view of pupa, showing the last larval molt skin and the depressed segmented area.

Fig. 4.—Ventral view of pupa.